

Claims

- SUB A4
1. A vector for increasing the copy number of plasmids, comprising a transposable element containing a moderate or high copy number origin of replication capable of *in vitro* transposition into a target plasmid.
2. The vector of claim 1 wherein the transposable element further comprises a transcription control sequence.
3. The vector of claim 2 wherein the transcription control sequence is the T7 promoter.
4. The vector of claim 1 wherein the origin of replication is the colE1 ori.
5. The vector of claims 1 or 2, further comprising an antibiotic resistance gene.
6. The vector of claim 5, wherein the antibiotic resistance gene is a kanamycin resistance gene.
7. The vector of claim 1 or 2, further comprising a counterselectable marker.
8. The vector of claim 7, wherein the counterselectable marker is the *sacB* gene from *B. subtilis*.
9. A vector for increasing the copy number of plasmids comprising:
(a) a transposable element containing a moderate or high copy number origin of replication;
(b) an antibiotic resistance gene; and
(c) a counterselectable marker.
10. The vector of claim 9, wherein the transposable element further comprises a transcription control sequence.
11. A vector for increasing the copy number of plasmids comprising:
- SUB A5
- SUB A6

SUB
A1

- (a) a transposable element containing a pBR322 origin of replication;
(b) a kanamycin resistance gene; and
(c) a *B. subtilis* *sacB* gene.

5 12. The vector of claim 11, further comprising a T7 promoter.

13. The vector of claim 12, which is pTRANS-SacB.

SUB
A1

14. A vector for increasing the copy number of plasmids comprising:
(a) a transposable element containing a moderate or high copy number origin of replication;
(b) an antibiotic resistance gene; and
(c) a transcription control sequence.

15 15. The vector of claim 14, which is pTRANS.

SUB
A1

16. An improved BAC vector comprising a high copy origin of replication flanked by cleavage sites for a restriction enzyme, wherein cleavage of the vector with the restriction enzyme leaves single base extensions for cloning and removes the high copy origin of replication.

20

17. The vector of claim 16, further comprising a BST X1 site.

18. The vector of claim 17, which is pBacTA.PUC2.

25

19. A method for increasing the copy number of a target plasmid comprising: mixing, in vitro, the target plasmid with the vector of any of claims 1, 2, 9, 11, 13 or 14 under conditions permitting introduction of the moderate or high copy number origin of replication into the target plasmid.

30

20. A method for sequencing a gene in a low copy number plasmid, comprising:
(a) mixing, in vitro, the target plasmid with the vector of any of claims 1, 2, 9, 11, 13 or 14 under conditions permitting introduction of the moderate or high copy number ori into the target plasmid;

- (b) transforming the mixture into cells;
- (c) selecting transformants for sequencing; and
- (d) determining the sequence of the gene.

5 21. A method for screening for BAC clones containing a transposable element comprising:

(a) mixing, in vitro, the BAC clone with the vector of any of claims 1, 2, 9, 11, 13 or 14 under conditions permitting introduction of the moderate or high copy number ori into the target plasmid;

10 (b) transforming the mixture into cells;

(c) detecting a phenotypic change in the clones transformed with the mixture relative to clones transformed with BAC vector alone.

15 22. The method of claim 21, wherein the phenotypic change is an increase in gene expression.

23. The method of claim 21, wherein the phenotypic change is a decrease in gene expression.

20 24. A method for increasing expression of a gene in a target plasmid comprising:

(a) mixing, in vitro, the target plasmid with the vector of any of claims 2, 10 or 12-15 under conditions permitting introduction of the moderate or high copy number ori into the target plasmid;

25 (b) transforming the mixture into cells capable of recognizing the transcription control element and expressing the gene.

25. The method of claim 24, wherein the transcription control element is the T7 promoter and the cells express T7 polymerase.

30 26. A method for full length cloning of genes comprising

(a) providing a BAC library

(b) mixing, in vitro, the library with the vector of any of claims 2, 10 or 12-15;

(c) transforming the mixture into cells:

(d) isolating full-length genes from the cells.

- ~~generating
comprising
different fr
n of the c~~

add A^9

add A^{10}

The first part of the paper is devoted to the study of the asymptotic behavior of the solutions of the system (1.1) as $\epsilon \rightarrow 0$. In the second part, we study the asymptotic behavior of the solutions of the system (1.1) as $\epsilon \rightarrow 0$. In the third part, we study the asymptotic behavior of the solutions of the system (1.1) as $\epsilon \rightarrow 0$.